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Selective Brain Cooling During and After Prolonged Global Ischemia Reduces Cortical Damage in Rats

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Background and Purpose: Studies of the cerebroprotective effects of selective brain cooling have failed to show amelioration of ischemic injury in the cerebral cortex. This study was designed to test the hypothesis that mild-to-moderate selective brain cooling initiated after the onset of global brain ischemia in rats protects the cerebral cortex and improves neurological outcome.

Methods: Global forebrain ischemia for 30 minutes in 27 fasted adult male Wistar rats was achieved by bilateral carotid occlusion and hypotension. In group 1, brain temperature, measured in the temporalis muscle, was maintained at 37–38°C throughout the experiment. In group 2, brain temperature fell spontaneously during ischemia to 34.7±0.1°C and rose spontaneously to 36–37°C after 10 minutes of recirculation. In group 3, brain temperature was lowered with ice packs placed around the head after 15 minutes of ischemia to 24.1±0.9°C by the end of ischemia, maintained at 30.0±1.0°C for the first hour of recirculation, then allowed to rise to 36–37°C.

Results: Seven-day survival was 0% (0 of 6) in group 1, 73% (8 of 11) in group 2, and 100% (6 of 6) in group 3. Severity of neuronal damage was less in group 2 than in group 1 in the cortex ($p<0.05$) and hippocampal CA1 ($p<0.05$) and CA3 regions ($p<0.05$). Group 3 had less neuronal damage than group 2 in both cortex ($p<0.02$) and striatum ($p<0.02$). Furthermore, postischemic weight loss was less and neurobehavioral scores were significantly higher in group 3.

Conclusions: This study shows that selective brain cooling increases survival from prolonged global ischemia and reduces neuronal injury in the cerebral cortex as well as the striatum and hippocampus. (*Stroke* 1992;23:1792–1797)

KEY WORDS • cerebral ischemia • hypothermia • neuronal damage • rats

Recently there has been a renewal of interest in the protective effects of hypothermia during and after ischemic insults to the brain. This has come about mainly because it has been shown that small reductions in brain temperature markedly protect the brain from ischemic injury.¹ Investigators have demonstrated that mild hypothermia initiated as late as 5 minutes after a 10-minute period of forebrain ischemia in rats lessens ischemic damage in the striatum and hippocampus.² Two studies have demonstrated that mild hypothermia protects the cerebral cortex from ischemic damage,^{3,4} and in each of these studies there was concurrent lowering of body temperature. Total body cooling produces deleterious systemic effects and for this reason has largely been abandoned as a therapeutic option for patients with brain injury. In its place has emerged selective brain cooling (SBC), which is the selective reduction of brain temperature while maintaining normal body temperature. The present study is

unique in that we examined the cerebroprotective effects of SBC in rats undergoing severe ischemia for a prolonged period (30 minutes), from which it is rare to

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have survivors under normothermic conditions. We tested the hypothesis that mild selective brain cooling initiated after the onset of global ischemia in rats protects the cerebral cortex and that more aggressive brain cooling further protects the cortex and improves neurological outcome.

Materials and Methods

All animal protocols were reviewed and approved by the Animal Care Committee of the University of California, San Francisco.

Fasted adult male Wistar rats (Simonsen, Gilroy, Calif.) weighing 310±4 g (mean±SEM) were anesthetized with 3% halothane, intubated, and ventilated with a rodent ventilator (Harvard model 683, South Natick, Mass.) to achieve normal blood gases, measured using a Corning model 178 blood gas analyzer (Corning, Medfield, Md.). Anesthesia was maintained using 0.5% halothane, a 2:1 mixture of N₂O and O₂, and pancuronium bromide for paralysis. The femoral vessels were catheterized for blood pressure monitoring, blood sampling, hemorrhage, and drug administration. The arterial catheter was connected to a pressure transducer

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that was calibrated prior to each experiment. Precalibrated thermocouple probes were placed in the temporalis muscle adjacent to the parietal bone (Malinckrodt, St. Louis, Mo.) and in the rectum (YSI model 43TA, Yellow Springs, Ohio). In addition, the correlation between temporalis muscle and striatal temperatures during and after ischemia was assessed in five passively cooled and six actively cooled rats. Striatal temperature was measured with a 33-gauge thermocouple probe (model HYP-0-33, Omega, Stamford, Conn.) tunneled subcutaneously under an intact scalp and inserted into the brain through a burr hole. Position of the probe was confirmed at autopsy. Temperatures were monitored continuously and recorded periodically (Mon-a-Therm 6500, Malinckrodt). Continuous electroencephalographic monitoring (model 78208A, Hewlett-Packard Co.) was performed with electrodes placed in the temporalis muscle and grounded to the ear. Through a midline neck incision, the carotid arteries were dissected free of surrounding nerves and fascia and encircled loosely with thread. Halothane was discontinued 30 minutes before ischemia, and 50 units heparin was given intravenously. Before ischemia, the head and rectal temperatures were maintained at 37–38°C with heating lamps. Global brain ischemia was achieved using common carotid occlusion and hypotension.^{4,5} With the rat in the supine position, the mean arterial pressure (MAP) was lowered with 2.5 mg i.v. trimethopran and withdrawal of blood into a syringe. The shed blood was kept at 37–38°C with preheated water bags. When the MAP reached 70–80 mm Hg, the carotid arteries were occluded with Teflon-coated vascular clamps (model ST-B-1A, Accurate Surgical Scientific Instruments, Westbury, N.Y.). The MAP was then lowered to 50 mm Hg, marking the start of ischemia, and maintained at 50±5 mm Hg for 30 minutes by withdrawal and infusion of blood. Rats were excluded from data analysis if the electroencephalogram did not become isoelectric or if the blood pressure could not be controlled at the desired level. After 30 minutes of ischemia, the carotid clamps were removed, and the vessels were inspected for patency. The shed blood was reinfused over 2 minutes, and 0.5 mEq sodium bicarbonate was given to counteract metabolic acidosis. Nitrous oxide was discontinued 15 minutes after the ischemic period. Rats were extubated when they exhibited strong respiratory activity, usually 60–90 minutes after the ischemic period. After 2 hours of reperfusion, the catheters, electrodes, and temperature probes were removed, and the incisions were closed. The animals were returned to their cages and given food and water ad libitum. Sham-operated rats underwent the same protocol, excluding carotid clamping but including 30 minutes of hemorrhagic hypotension.

Four groups of animals were studied: sham-operated control rats ($n=4$); group 1, normothermic brain temperature ($n=7$); group 2, mildly hypothermic brain temperature by passive cooling ($n=13$); and group 3, moderately hypothermic brain temperature by active cooling ($n=7$). In all groups, rectal temperature was maintained at 37–38°C with a heating lamp throughout the experiment. In group 1 and the sham-operated control group, brain temperature was maintained at 37–38°C throughout the experiment. In group 2, brain temperature was allowed to fall passively during ischemia and rise spontaneously dur-

ing recirculation. In group 3, brain temperature fell passively during the first 15 minutes of ischemia; active cooling was then initiated by placing ice bags around the head and neck. Active cooling was continued for the first hour of recirculation, after which brain temperature was allowed to rise spontaneously.

Rats were observed closely during the first 24 hours for seizure activity and signs of neurological deterioration. They were killed if they developed intractable seizures or lost the righting reflex; in our experience, this signals impending death. The rats underwent a daily weight measurement and neurobehavioral examination that consisted of a 15-point scoring system based on gross motor function, level of consciousness, grooming, feeding activity, and reaction to noise and tail pinching. Rats that died were excluded from analysis of weight gain and neurobehavioral score beginning on the day of death. After a 7-day survival period (or sooner if seizures or coma developed), the rats were reanesthetized with halothane for transcardiac perfusion-fixation of the brain. A 2-minute rinse with heparinized lactated Ringer's solution was followed by a 5-minute infusion of 10% buffered formalin under 135 cm H₂O pressure. Brains were immediately removed and stored in 10% buffered formalin for at least 1 week. They were then embedded in paraffin, and 8- μ -thick serial coronal sections of the forebrain were taken and stained with hematoxylin-eosin. Brain injury was assessed in a semi-quantitative manner based on severity of neuronal damage and presence of necrosis. Nuclear pyknosis, cytoplasmic eosinophilia, and cytological deformity were the minimum criteria accepted for ischemic cell change. Other advanced features of neuronal damage included incrustation, karyorrhexis, and cell loss in areas of necrosis. Severity of neuronal damage was expressed as the ratio of damaged neurons to total neurons: grade 0, less than 5%; grade 1, 5% to <25%; grade 2, 25% to <50%; grade 3, 50% to <75%; and grade 4, 75% and greater. Grading was performed by two investigators who were blinded to the temperature manipulations.

Statistical analysis was performed using the STATVIEW II software program (Abacus Concepts, Inc., Berkeley, Calif.). Mortality was compared using χ^2 analysis. For neurological scores and neuronal damage, Kruskal-Wallis testing was used. Blood pressure, blood gases, and daily weight changes were analyzed using paired t test and analysis of variance for repeated measures. Results were considered significant at $p<0.05$.

Results

Four rats were excluded because the electroencephalogram did not become isoelectric (one from group 1, two from group 2, and one from group 3) despite rapid lowering of MAP.

Arterial blood gases were not different between ischemic groups before or during ischemia (Table 1). MAP returned to its preischemic level by 5, 10, and 30 minutes of recirculation for the sham-operation group, group 2, and group 3, respectively. In group 1, MAP did not return to its preischemic level; however, it stabilized above 130 mm Hg after 15 minutes of recirculation (Table 1).

At the start of ischemia, mean brain temperature was 37.4–37.5°C in all groups. In group 2, brain temperature fell gradually to 34.7±0.1°C by the end of ischemia and

TABLE 1. Mean Arterial Pressure and Arterial Blood Gas Values Before, During, and 2 Hours After Ischemia

Group	n	pH	PaCO ₂ (mm Hg)	PaO ₂ (mm Hg)	Base excess	Mean arterial pressure (mm Hg)
Sham						
Before	4	7.39±0.02	41.5±3.2	127.8±8.7	-0.3±0.9	151±0.8
During	4	7.27±0.03*	38.5±5.3	134.5±10.4	-8.5±1.0†‡	50±5.0
After	4	7.37±0.02	46.0±3.9	72.3±5.0†	0.8±0.9	154±6.5
Group 1						
Before	6	7.36±0.01	40.2±1.4	116.7±5.6	-2.3±0.6‡	149±1.9
During	6	7.16±0.02†	34.3±3.8	140.3±7.2*	-15.8±1.5†	50±5.0
After	6	7.46±0.02†	31.7±1.3†	85.7±2.9†	0.2±1.0	132±2.8†‡
Group 2						
Before	11	7.36±0.02	38.3±1.4	134.1±8.3	-2.8±0.6§	147±2.8
During	10	7.21±0.03†	38.1±4.9	131.0±7.1	-13.0±0.9†	50±5.0
After	11	7.48±0.01†‡	29.1±1.0†‡	96.2±2.1†	0.4±0.5†	147±3.2
Group 3						
Before	6	7.40±0.02	40.5±1.9	130.8±8.5	0.2±0.7	143±4.5
During	5	7.17±0.03†	40.2±3.4	143.0±7.8	-13.4±1.2†	50±5.0
After	6	7.41±0.04	37.7±4.3	84.2±6.8†	-0.3±0.8	144±4.2

Values are mean±SEM. Two hours after ischemia, all animals were spontaneously breathing room air.

* $p<0.05$, † $p<0.01$ for within-group comparisons with values before ischemia.

‡ $p<0.05$, § $p<0.01$ for between-group comparisons with group 3.

recovered to its preischemic level by 15 minutes of recirculation (Figure 1). In group 3, brain temperature fell to $35.9\pm 0.1^\circ\text{C}$ after 15 minutes of ischemia. After application of the ice packs, brain temperature fell rapidly, reaching $24.1\pm 0.9^\circ\text{C}$ by the end of ischemia. During the first hour of recirculation, brain temperature in group 3 was $30.0\pm 1.0^\circ\text{C}$; it increased to baseline 30 minutes after removing the ice packs. Rectal temperature was maintained between 36.5 and 37.5°C in all rats except one in group 3, whose rectal temperature fell transiently to 35.6°C during the early recirculation period.

Overall, brain temperature measured in the temporalis muscle correlated well with striatal temperature

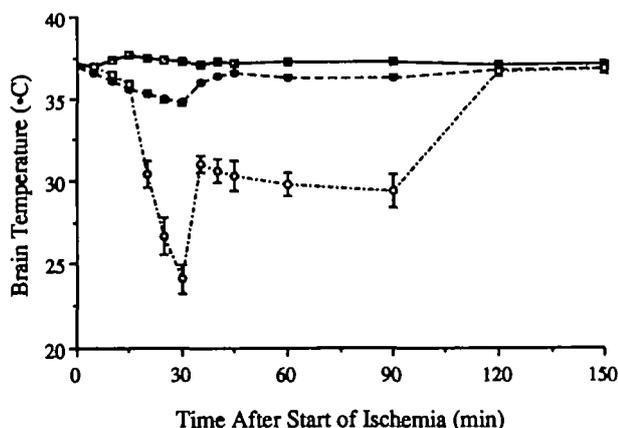


FIGURE 1. Brain temperature ($^\circ\text{C}$, measured in temporalis muscle) during and after 30 minutes of ischemia in normothermic group 1 ($-\square-$; $n=6$), passively cooled group 2 ($--\bullet--$; $n=11$), and actively cooled group 3 ($\cdots\circ\cdots$; $n=6$) rats. Data are mean±SEM.

except during early recirculation in group 3. The correlation coefficients for temporalis muscle and striatal temperatures were high for groups 2 and 3 ($r=0.97$ and 0.88 , respectively) during ischemia. During recirculation, the correlation coefficient remained high for group 2 ($r=0.88$) but was poor in group 3 ($r=0.36$). During ischemia in groups 1 and 2 and the sham-operated group, temporalis muscle temperature was 0.1 – 0.3°C higher than striatal temperature; however, during ice application in group 3, temporalis muscle temperature was 3 – 4°C lower than striatal temperature.

Mortality in group 1 was 100% and was significantly higher than in groups 2 (27%, $p<0.02$) and 3 (0%, $p<0.005$). The difference in mortality in groups 2 and 3 was not significant. One rat in group 1 and three in group 2 were found dead between the first and second day and were not included in the histological analysis. The remaining five in group 1 developed coma 12–18 hours after ischemia. Four of the five rats developed seizures and were immediately killed. Neurobehavioral scores were significantly higher in group 3 than in group 2 for the first 5 days after ischemia (Figure 2).

Compared with group 1, passive cooling decreased neuronal damage in group 2 in the cortex ($p<0.05$) and hippocampal CA1 ($p<0.05$) and CA3 regions ($p<0.05$) but not in the striatum (Figure 3). Compared with group 1, active cooling in group 3 reduced neuronal damage in all four regions. Compared to group 2, active cooling reduced neuronal damage in the cortex ($p<0.02$) and striatum ($p<0.02$).

On each day after ischemia, body weight (as a percentage of preischemic weight) was significantly lower in group 2 than in group 3 rats (Figure 4). Rats in the sham-operated group began eating and drinking 2–4 hours after

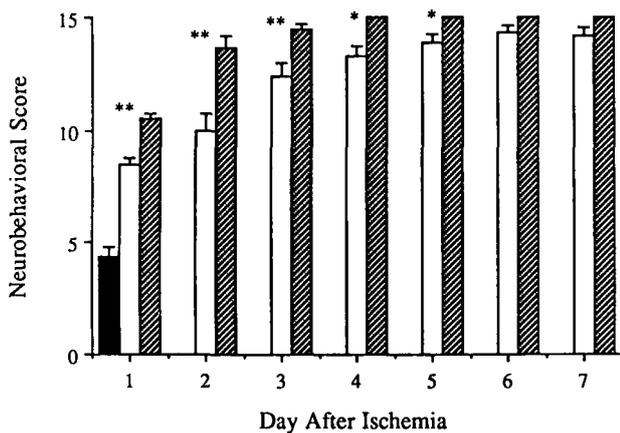


FIGURE 2. Neurobehavioral scores (normal score, 15) on successive days after 30 minutes of ischemia in normothermic group 1 (filled bar; $n=5$), passively cooled group 2 (open bars; $n=8$), and actively cooled group 3 (hatched bars; $n=6$) rats. Data are mean \pm SEM. Group 2 vs. group 3: * $p \leq 0.02$, ** $p \leq 0.005$.

returning to their cages. They gained weight and appeared neurologically normal by the first postoperative day.

Discussion

In the present study we sought to challenge the potential for cerebroprotection by hypothermia by extending the duration of ischemia to 30 minutes and by instituting SBC 15 minutes after the onset of ischemia. Our results show that a mild reduction of 2–3°C in brain temperature during ischemia improves survival and lessens neuronal damage after prolonged forebrain ischemia in rats. In addition, more aggressive brain cooling further improves neurological outcome and reduces neuronal damage in regions of the brain that were either partially or not at all protected by mild hypothermia. Most investigators^{1,2,5,6} have been unable to demonstrate cortical protection by hypothermia because shorter durations of ischemia do not consistently produce cortical injury. Our results suggest that the cortex not only responds favorably but does so to a

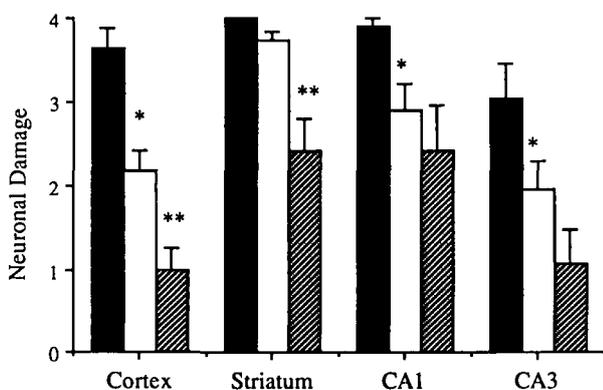


FIGURE 3. Severity of neuronal damage by region graded as necrotic neurons/total neurons: 0, <5%; 1, 5% to <25%; 2, 25% to <50%; 3, 50% to <75%; and 4, $\geq 75\%$. Data are mean \pm SEM. Filled bars, group 1 ($n=5$); open bars, group 2 ($n=8$); and hatched bars, group 3 ($n=6$). Group 1 vs. group 2, * $p < 0.05$; group 2 vs. group 3, ** $p < 0.02$.

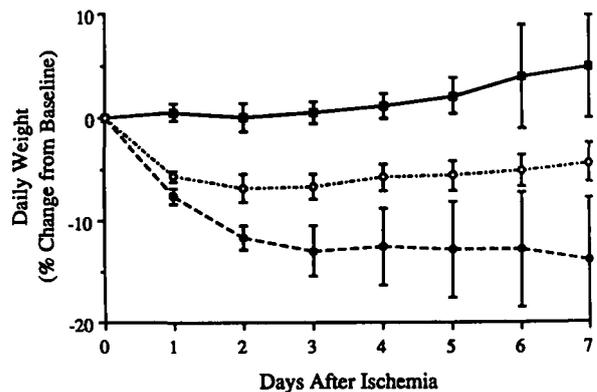


FIGURE 4. Daily weight changes as percentage of preischemic weight on successive days after 30 minutes of ischemia in sham-operated (—■—; $n=4$), passively cooled (—○—; $n=8$), and actively cooled (—△—; $n=6$) rats. Data are mean \pm SEM. At all time points, values were significantly different between the three groups.

greater extent than the hippocampus and striatum. Furthermore, these histopathologic differences persisted throughout a 7-day survival period.

It has been known for over 40 years that hypothermia protects the brain from ischemic injury.^{7,8} Mechanisms of this protection include reductions in metabolism,^{9,10} intracellular acidosis,^{11,12} and edema formation,^{13–15} as well as an increase in the rate of resolution of acidosis during recirculation.¹¹ In addition, Busto et al¹⁶ showed that the rise in extracellular glutamate concentration during ischemia was markedly attenuated in rats whose brain temperature was lowered to 33°C immediately after starting ischemia. The weakly protective effect of SBC in the hippocampus in the present study may have resulted from the gradual onset of cooling, which could have permitted a rise in glutamate during the first part of the ischemic period. If this were true, it would suggest that SBC is unable to reverse the effects of glutamate once its concentration reaches a critical level.

The greatest protection occurred in the cortex, where neuronal damage was reduced in group 2 to 60% and in group 3 to 28% of the damage observed in group 1. However, the cortical protection was not complete. One rat in group 3 had grade 4 damage in one cortical hemisphere, and several others had one or two small cortical infarcts. These findings concerning the incompleteness of the cortical protection by hypothermia are consistent with those of Minamisawa et al,³ who observed scattered cortical infarcts and neuronal necrosis in rats whose brain temperature fell to 32°C during 15 minutes of forebrain ischemia. Based on the stepwise reduction of neuronal damage with decreasing temperatures observed in groups 2 and 3, however, it seems logical that more aggressive brain cooling (either to lower temperatures or for longer durations) might completely prevent ischemic damage in the cortex.

Of the other three regions examined in this study, we found that striatal damage was reduced only by active cooling. The CA1 and CA3 regions of the hippocampus were protected by mild cooling, but further reduction of brain temperature did not provide additional protection. In contrast, Busto et al¹ observed significant reductions in the number of ischemic cells in the

striatum at 34°C and no ischemic cells at 30°C after 20 minutes of four-vessel occlusion in rats. In that study, the hippocampus was also more protected at lower temperatures, although neuronal loss was not completely prevented even at 30°C. The discrepancy between their results and ours is likely due to the differences in the rate of temperature reduction during ischemia as well as the shorter duration of ischemia in their study. Busto et al¹ recorded a 6–7°C reduction of brain temperature during 20 minutes of ischemia. The head of the animal was positioned off the ground and away from the body in a stereotaxic device. In addition, the calvarium was exposed. This created a favorable situation for heat loss from the brain. Striatal temperature spontaneously fell to 32°C between 5 and 10 minutes of ischemia. In the present study, rats were in the supine position with their heads flat against the surgical table, as might occur clinically. Furthermore, the scalp was intact. After 15 minutes of ischemia, brain temperature had fallen to only 35.6±0.1°C and 35.9±0.1°C in groups 2 and 3, respectively. Thus, for at least the first 10 minutes, animals in the present study underwent essentially normothermic ischemia. This, together with the 30-minute duration of ischemia, accounted for the presence of neuronal injury throughout all four regions examined despite the reduction in brain temperature. With this pattern of cooling, it appears that significant neuronal injury had occurred before a protective temperature had been reached.

It is also important to recognize that in a parallel group of actively cooled rats, the temporalis muscle temperature underestimated striatal temperature by as much as 4°C, and during application of ice packs in the early recirculation period there was a very poor correlation between the two temperatures. It is possible that the incompleteness of the protection by hypothermia in group 3 was due to insufficient brain cooling in some animals.

Another likely explanation for the regional differences in protection by SBC is the possible existence of temperature gradients within the brain. This method of producing forebrain ischemia results in severe reductions of blood flow in the cortex, hippocampus, and striatum; however, Smith et al^{17,18} have shown that the reduction of blood flow to deeper brain structures such as the thalamus is considerably less severe. This would lead to the existence of temperature gradients, with the core being warmer than the outer regions of the brain. These gradients are likely to be greater when SBC is achieved by external means of evaporative and conductive heat losses as occurred in this study. Because the cortex is the outermost region, it probably cooled faster and to a lower temperature than the hippocampus and striatum.

Finally, SBC may protect the brain through effects on cerebral blood vessels. It has been recently documented both in vitro¹⁹ and in vivo²⁰ that hypothermia decreases cerebral vascular resistance. Kuluz et al²⁰ measured a twofold increase in cerebral blood flow in nonischemic rats when brain temperature was lowered to 33.4°C by SBC. Baldwin et al²¹ found that hypothermia initiated after 20 minutes of global ischemia was associated with a longer hyperemic period. Thus, SBC may cause an increase in vasodilating compounds such as nitric oxide, prostaglandins, or adenosine that may exert a protective effect during ischemia and recirculation.

The patterns of temperature reduction were chosen to mimic the clinical situation of global brain ischemia that occurs during cardiac arrest and what might occur if SBC were accomplished in that setting. This study supports the idea that interventions designed to lower brain temperature during cardiac arrest may improve neurological outcome.

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